REMARKS

Regarding the amendments

Claim 6 has been amended to include the article "a" before "human SNAP-25." The dependency of claims 45 to 49 has been amended to correct dependency on several canceled claims. Claim 64, which recites "said acceptor fluorophore" has been amended to depend from claim 50, which provides antecedent basis for the term "acceptor fluorophore." In addition, claims 45 to 49 have been amended to recite "nanomole" instead of "nanomoles." Each of these amendments correct minor typographical errors or correct claim dependencies and do not add new matter.

Claim 59 has been amended to indicate that the acceptor included in the substrate is a "non-fluorescent acceptor." The amendment to claim 59 is supported throughout the specification, for example, at page 88, lines 9-12, which indicates that non-fluorescent acceptors include DABCYL and QSY® dyes.

As set forth above, each of the claim amendments is supported by the specification or claims as originally filed and does not add new matter. Accordingly, Applicants respectfully request that the Examiner enter the amendments.

Regarding the Objection to the Specification

The objection to the specification for using several trademarks is respectfully traversed. The Office Action indicates that, while the use of trademarks is permissible in

patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner that might adversely affect their validity as trademarks. The Office Action indicates that trademarks should be capitalized and accompanied by generic terminology.

Applicants respectfully submit that trademarks have not been used in the specification in a manner which would adversely affect their validity as trademarks. Rather, the proprietary nature of the marks has been respected by the use of the proper trademark symbol "®" following the name of the trademark. See MPEP 608.01(v). In particular, trademarks such as Alexa Fluor® 488, QSY 7® and BOTOX® have been indicated by the "®" trademark symbol. Given that the proprietary nature of the marks has been respected, Applicants submit that capitalization of the marks is not necessary.

Applicants further submit that accompanying generic terminology is unnecessary where the trademark has a fixed and definite meaning in the art, unless some characteristic of the trademarked article is involved in the invention (MPEP 608.01(v)). In the present case, the trademarks Alexa Fluor® 488, QSY 7® and BOTOX® each have a fixed and definite meaning in the art. Thus, the trademarks used in the specification have been properly used without accompanying generic terminology. Accordingly, Applicants respectfully request that the objection to the specification be removed.

Regarding the Objections to the Claims

The objections to claims 45 to 50 for several informalities are respectfully traversed. In particular, claims 45 to 50 depend from several canceled claims and recite "nanomoles" rather than "nanomole."

In view of the previous cancellation of claims 9 to 44, the dependency of claims 45 to 49 has been amended such that these claims depend on pending base claims 1, 2, 3 and 4. Furthermore, claims 45 to 49 also have been amended herein to recite "nanomole/minute/milligram toxin." In view of the above remarks and amendments, Applicants respectfully request that the Examiner withdrawn the objections to claims 45 to 50.

Regarding the double patenting rejection

The provisional rejection of claims 1 to 8 and 45 to 67 under the judicially created doctrine of obviousness-type double patenting as allegedly unpatentable over claims 1 to 6, 11, 13 to 15 and 31 to 38 of copending application Serial No. 10/261,161 is respectfully traversed. Applicants respectfully defer responding to this provisional rejection until allowable subject matter is indicated.

Regarding the rejections under 35 U.S.C. § 112, second paragraph

The rejection of claims 1 to 8 and 45 to 67 under 35 U.S.C. §112, second paragraph, as allegedly lacking clarity respectfully is traversed.

Regarding the phrase "under the appropriate conditions"

The concluding phrase of claim 1 recites that "under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor." The Office Action alleges that the metes and bounds of such "appropriate conditions" do not appear to be set forth or defined in the specification. Applicants respectfully traverse this ground for rejection.

Applicants submit that, in view of the specification, one skilled in the art understands that resonance energy transfer can be exhibited between a particular donor fluorophore/acceptor pair. As is well known in the art and set forth in the specification, fluorescence resonance energy transfer is a physical process by which energy is transferred non-radiatively from an excited donor fluorophore to an acceptor through intramolecular long-range dipole-dipole coupling (specification at page 68, lines 21-26). As further taught in the specification, actual resonance energy transfer is exhibited when the donor fluorophore is excited within its absorbance spectrum and where there is an appropriate separation distance and orientation of the donor fluorophore and acceptor as described by the Forster equation. The extent of actual

resonance energy transfer further depends in part on the fluorescent quantum yield of the donor fluorophore and its energetic overlap with the acceptor (page 68, lines 21-33; page 69, line 25, to page 70, line 71, line 19).

In view of what was known about the phenomenon of resonance energy transfer and the teachings of the specification, it is clear to the skilled person that "appropriate conditions" for resonance energy transfer include excitation of the donor fluorophore where a donor fluorophore/acceptor pair has been selected such that there is substantial spectral overlap between the emission spectrum of the donor fluorophore and the excitation spectrum of the acceptor and where the donor fluorophore and acceptor have been positioned in the clostridial toxin substrate with an appropriate separation distance and orientation. In sum, Applicants submit that the clause "under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor" is clear to the skilled person in view of the specification. Applicants therefore respectfully request that the Examiner remove this ground for rejecting the claims as allegedly indefinite.

Regarding several trademarks

Claims 54 and 59 are allegedly indefinite due to the use of the trademarks Alexa Fluor® 488 and QSY® 7 in the absence of sufficiently detailed descriptive generic terminology. In this regard, the Office Action opines that products denoted by trademark names are subject to change.

Applicants respectfully submit that the products represented by the Alexa Fluor® 488 and QSY® 7 trademarks are well known products produced with consistent characteristics by their manufacturers. Thus, detailed generic terminology for these trademarked products should not be required. Nevertheless, reference to the trademarked product QSY® 7 has been deleted from claim 59, and claim 54 has been canceled in order to further prosecution of the subject application. In view of the above, Applicants respectfully request that the Examiner reconsider and remove this ground for rejecting claims 54 and 59.

Regarding the phrase "can be cleaved"

Claims 45 to 49 also stand rejected as allegedly vague and indefinite due to the phrase "can be cleaved." The Examiner rejects these claims on the ground that it is unclear whether cleavage does or does not occur.

Applicants submit that claims 45 to 49, which are directed to a clostridial toxin substrate which "can be cleaved" with an activity of at least 1, 20, 50, 100 or 150 nanomole/minute/ milligram toxin, respectively, are clear and definite to the skilled person. In particular, in view of the specification and what was well known in the art at the time the application was filed, one skilled in the art understands that the rate of cleavage per milligram toxin will be dependent on the reaction conditions. In particular, the skilled person understands that the activity of proteases depends on conditions including the buffer, pH, salt concentration, temperature, and presence and concentration of any necessary co-factors. The

specification teaches, for example, that conditions suitable for clostridial toxin protease activity generally include an appropriate concentration of a buffer such as HEPES, Tris or sodium phosphate; a reducing agent such as β-mercaptoethanol or dithiothreitol (DTT) where the sample contains dichain toxin; and a source of zinc while generally excluding zinc chelators such as EDTA (page 102, line 22, to page 103, line 15). The specification further teaches that exemplary conditions suitable for BoNT/A protease activity can be incubation at 37°C in a buffer such as 30 mM HEPES (pH 7.3) containing a reducing agent such as 5 mM dithiothreitol; a source of zinc such as 25 μM zinc chloride and 1 μg/ml toxin, with bovine serum albumin (BSA) in the range of 0.1 mg/ml to 10 mg/ml (page 105, line 29, to page 106, line 6; see, also, Example I). In view of the above, it is clear that the phrase "can be cleaved" refers to the cleavage that will occur in the presence of active toxin and under conditions which support the activity of a zinc metalloprotease.

In sum, Applicants submit that claims 45 to 49 are clear and definite as written and, therefore, respectfully request that the Examiner remove the rejection of these claims under 35 U.S.C. §112, second paragraph.

Regarding antecedent basis

The Office Action indicates that there is insufficient antecedent basis for the term "human SNAP-25" in claim 5 and for the term "said acceptor fluorophore" in claims 64 to 67. Given that claim 5 does not include the term "human SNAP-25," Applicants address this rejection as it pertains to claim 6.

Applicants submit that claims 6 and 64 to 67 are clear and definite as written. Nevertheless, in order to further prosecution of the subject application, claim 6 has been amended to more clearly indicate that the recited human SNAP-25 is "a human SNAP-25." Furthermore, claim 64 has been amended to depend from claim 50, which provides antecedent basis for the term "acceptor fluorophore." In view of the above, Applicants respectfully request that these grounds for rejection be removed.

Regarding the rejection under 35 U.S.C. § 103

The rejection of claims 1 to 8 and 45 to 67 under 35 U.S.C. §103(a) as allegedly unpatentable over Holskin et al. in view of Ekong et al. is respectfully traversed.

The Office Action alleges that Holskin et al. describe substrates containing a donor fluorophore, acceptor fluorophore and a specific protease cleavage site. Holskin et al. further allegedly report the donor/acceptor pair EDANS/DABCYL and that this donor/acceptor pair has been successfully used in fluorescence-based assays to detect HIV protease and renin protease activity. While the Office Action acknowledges that Holskin et al. do not teach a BoNT/A or other clostridial toxin substrate, the cited publication by Ekong et al. allegedly remedies this deficiency by describing an effective BoNT/A substrate for *in vitro* assays.

Applicants assert that the clostridial toxin substrates of the invention are unobvious over the cited references by Holskin et al. and Ekong et al., both alone and in

combination. Specifically, neither Holskin et al. nor Ekong et al. teach or suggest a clostridial toxin substrate containing a BoNT/A recognition sequence that includes a cleavage site which intervenes between a donor fluorophore and an acceptor, where fluorescence resonance energy transfer occurs under the appropriate conditions. Holskin et al. relates generally to fluorescence resonance energy transfer (FRET) but does not teach or suggest a BoNT/A substrate suitable for a FRET assay. Furthermore, the cited reference by Ekong et al. at best describes an *in vitro* assay for BoNT/A activity using an antibody specific for the BoNT/A cleavage product. The substrate of Ekong et al. does not include a donor fluorophore and an acceptor, and one skilled in the art would not have been motivated to modify the BoNT/A substrate of Ekong et al. to incorporate a donor fluorophore and acceptor suitable for fluorescence resonance energy transfer. Specifically, Ekong et al. report that a segment of SNAP-25 (residues 134-206) expressed as a maltose-binding protein (MBP) fusion protein and isolated by affinity chromatography following cleavage can serve as a BoNT/A substrate in an *in vitro* endopeptidase assay (Ekong et al., page 3337, abstract; see, also, Figure 1). Using antibodies specific for BoNT/A-cleaved SNAP-25, an enzyme-linked immunoassay (ELISA) with microtitre plates containing SNAP-25-MBP or the SNAP-$25_{134-206}$ peptide substrate was used to determine the activity of therapeutic preparations of BoNT/A (page 3339, "Preparation and characterization of antipeptide antibodies," and page 3343, "Assessment of the proteolytic activity of therapeutic BoNT/A"). Thus, Ekong et al. report that BoNT/A activity can be readily detected *in vitro* using a BoNT/A

substrate that has not been modified to include a donor fluorophore and acceptor.

Furthermore, Ekong et al. teach away from the claimed invention by emphasizing the importance of immunodetection to specificity of their BoNT/A assay. In particular, the authors indicate that an advantage of their assay is specificity resulting from specific cleavage coupled with specific immunodetection of the cleavage product (see page 3346, first sentence of first full paragraph). See, also, the second full paragraph of page 3346, in which the authors state that [i]n this study we have described a simple microtitre-based assay which utilizes a recombinant fragment of SNAP-25, spanning the BoNT/A toxin cleavage site as substrate, *together with targeted anti-peptide antibodies for measuring BoNT/A peptidase activity* in vitro (emphasis added). Thus, Ekong et al., asserting the importance of immunodetection to BoNT/A assay specificity, teach away from the claimed invention. In addition, one skilled in the art would not have known whether addition a bulky donor fluorophore and acceptor to a BoNT/A substrate would interfere with BoNT/A protease activity, and, therefore, further would not have been motivated to modify the proven Ekong et al. assay by substituting fluorescence resonance energy transfer (FRET)-based detection for immunodetection.

In sum, neither of the cited references, either alone or in combination, teach or suggest a BoNT/A substrate containing a donor fluorophore, an acceptor and a BoNT/A recognition sequence which includes a cleavage site.

Furthermore, there is no motivation to combine the teachings of Holskin et al. with those of Ekong et al. in order to modify Ekong et al.'s proven immunodetection-based assay. Absent such motivation, Applicants submit that the claimed clostridial toxin substrates are unobvious over the cited references. Accordingly, Applicants respectfully request that the Examiner reconsider and remove the rejection under 35 U.S.C. §103 of claims 1 to 8 and 45 to 67 as allegedly obvious over Holskin et al. in view of Ekong et al.

CONCLUSION

Applicants respectfully request that the Examiner consider the amendments and remarks herein above. The Examiner is invited to call the undersigned agent or Cathryn Campbell if there are any questions.

Respectfully submitted,

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